Claims

Sub C2

1. Process for the production of S-layer proteins
w h e r e i n
(a) a gram-negative prokaryotic host cell is provided which is transformed with a nucleic acid coding for an S-layer protein which is selected from
(i) a nucleic acid which comprises the nucleotide sequence from position 1 to 3684 shown in SEQ ID NO.1 optionally without the signal peptide-coding section,
(ii) a nucleic acid which comprises a nucleotide sequence corresponding to the nucleic acid from (i) within the scope of the degeneracy of the genetic code and
(iii) a nucleic acid which comprises a nucleotide sequence which hybridizes with the nucleic acids from (i) or/and (ii) under stringent conditions;
(b) the host cell is cultured under conditions which lead to an expression of the nucleic acid and to production of the polypeptide coded by it and
(c) the resulting polypeptide is isolated from the host cell.

2. Process as claimed in claim 1,
w h e r e i n
an E. coli host cell is used.

3. Process as claimed in claim 1 ~~or 2,~~
w h e r e i n
the polypeptide is isolated from the interior of the host cell in the form of an assembled S-layer structure.

4. Process as claimed in ~~one of the claims 1 to 3,~~ claim 1
w h e r e i n
the nucleic acid coding for the S-layer protein contains one or several insertions which code for peptide or polypeptide sequences.

5. Process as claimed in claim 4,
w h e r e i n
the insertions are selected from nucleotide sequences which code for cysteine residues, regions with several charged amino acids or Tyr residues, DNA-binding epitopes, metal-binding epitopes, immunogenic epitopes, allergenic epitopes, antigenic epitopes, streptavidin, enzymes, cytokines or antibody-binding proteins.

6. Process as claimed in claim 5,
w h e r e i n
the insertions code for streptavidin.

7. Process as claimed in claim 5,
w h e r e i n
the insertions code for immunogenic epitopes from herpes viruses, in particular herpes virus 6 or FMDV.

8. Process as claimed in claim 5,
w h e r e i n
the insertions code for enzymes such as polyhydroxybutyric acid synthase or bacterial luciferase.

9. Process as claimed in claim 5,
w h e r e i n
the insertions code for cytokines such as interleukins, interferons or tumour necrosis factors.

10. Process as claimed in claim 5,
w h e r e i n
the insertions code for antibody-binding proteins such as protein A or protein G.

11. Process as claimed in claim 5,
w h e r e i n
the insertions code for antigenic epitopes which bind cytokines or endotoxins.

12. Process as claimed in claim 5,
w h e r e i n
the insertions code for metal-binding epitopes.

13. Process as claimed in ~~one of the claims 1 to 12,~~ claim 1,
w h e r e i n
a nucleic acid coding for a gram-positive signal peptide is arranged in operative linkage at the 5' side of the nucleic acid coding for the S-layer protein.

14. Process as claimed in claim 13,
w h e r e i n
the nucleic acid coding for the signal peptide comprises
(a) the signal peptide-coding section of the nucleotide sequence shown in SEQ ID NO.1,
(b) a nucleotide sequence corresponding to the sequence from (a) within the degeneracy of the genetic code or/and
(c) a nucleotide sequence that is at least 80 % homologous to the sequences from (a) or/and (b).

15. Nucleic acid that codes for a recombinant S-layer protein and is selected from
(i) a nucleic acid which comprises the nucleotide sequence from position 1 to 3684 shown in SEQ ID NO.1 optionally without the signal peptide-coding section,
(ii) a nucleic acid which comprises a nucleotide sequence corresponding to the nucleic acid from (i) within the scope of the degeneracy of the genetic code and
(iii) a nucleic acid which comprises a nucleotide sequence which hybridizes with one of the nucleic acids from (i) or/and (ii) under stringent conditions,
wherein the nucleic acid contains at least one peptide or polypeptide-coding insertion within the region coding for the S-layer protein.

16. Nucleic acid as claimed in claim 15,
w h e r e i n
the insertion site is located at position 582, 878,

917, 2504 or/and 2649 of the nucleotide sequence shown in SEQ ID NO.1.

17. Vector,
w h e r e i n
it contains at least one copy of a nucleic acid as claimed in claim 15 ~~or 16.~~

~~18.~~ Cell,
w h e r e i n
it is transformed with a nucleic acid as claimed in claim 15 or 16 or with a vector as claimed in claim 17.

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19. Cell as claimed in claim 18,
w h e r e i n
it is a gram-negative prokaryotic cell and in particular an E. coli cell.

20. Cell as claimed in claim 18 ~~or 19,~~
w h e r e i n
it contains a recombinant S-layer structure.

21. Recombinant S-layer protein,
w h e r e i n
it is coded by a nucleic acid as claimed in claim 15 ~~or 16~~.

22. Recombinant S-layer structure,
w h e r e i n
it contains at least one protein as claimed in claim 21 as a subunit.

23. S-layer structure as claimed in claim 22,
w h e r e i n
it additionally contains at least one unmodified S-layer protein as a subunit.

24. S-layer structure as claimed in claim 22 ~~or 23,~~
w h e r e i n
it comprises several layers which are linked covalently or by affinity binding.

25. Use of an S-layer protein as claimed in claim 21 or an S-layer structure as claimed in one of the claims 22 to 24 as a vaccine or adjuvant.

26. Use as claimed in claim 25,
w h e r e i n
the vaccine or adjuvant additionally comprise a bacterial ghost which optionally contains further immunogenic epitopes in its membrane.

27. Use of an S-layer protein as claimed in claim 21 or an S-layer structure as claimed in one of the claims 22 to 24 as an enzyme reactor.

28. Nucleic acid which codes for an S-layer protein and is selected from
(i) a nucleic acid which comprises the nucleotide sequence from position 1 to 2763 shown in SEQ ID NO.5 optionally without the signal peptide-coding section,
(ii) a nucleic acid which comprises a nucleotide sequence corresponding to the nucleic acid from (i) within the scope of the degeneracy of the genetic code and

(iii) a nucleic acid which comprises a nucleotide sequence that hybridizes with the nucleic acids from (i) or/and (ii) under stringent conditions.

29. Nucleic acid as claimed in claim 28,
w h e r e i n
it contains at least one peptide-coding or polypeptide-coding insertion within the region coding for the S-layer protein.

30. Vector,
w h e r e i n
it contains at least one copy of a nucleic acid as claimed in claim 28 ~~or 29~~.

31. Cell,
w h e r e i n
it is transformed with a nucleic acid as claimed in claim 28 or 29 or with a vector as claimed in claim 30.

32. Cell as claimed in claim 31,
w h e r e i n
it contains a recombinant S-layer structure.

33. S-layer protein,
w h e r e i n
it is coded by a nucleic acid as claimed in claim 29.

34. Recombinant S-layer structure,
w h e r e i n
it contains at least one recombinant S-layer protein as a subunit which is coded by a nucleic acid as claimed in claim 29.

35. Use of an S-layer protein as claimed in claim 33 or of an S-layer structure as claimed in claim 34 as a vaccine or adjuvant.

36. Use of an S-layer protein as claimed in claim 33 or an S-layer structure as claimed in claim 34 as an enzyme reactor.

37. Process for the production of recombinant S-layer proteins,
w h e r e i n
(a) a host cell is provided which contains a nucleic acid coding for an S-layer protein which contains a peptide-coding or polypeptide-coding insertion within the region coding for the S-layer protein,
(b) the host cell is cultured under conditions which lead to an expression of the nucleic acid and to production of the polypeptide coded by it and
(c) the resulting polypeptide is isolated from the host cell or from the culture medium.

38. Process as claimed in claim 37,
w h e r e i n
the nucleic acid coding for the recombinant S-layer protein is selected from
(i) a nucleic acid which comprises the nucleotide

sequence from position 1 to 3684 shown in SEQ ID NO.1 optionally without the signal peptide-coding section,

(ii) a nucleic acid which comprises a nucleotide sequence corresponding to the nucleic acid from (i) within the scope of the degeneracy of the genetic code and

(iii) a nucleic acid which comprises a nucleotide sequence which hybridizes with one of the nucleic acids from (i) or/and (ii) under stringent conditions

39. Process as claimed in claim 37,
w h e r e i n
the nucleic acid which codes for the recombinant S-layer protein is selected from

(i) a nucleic acid which comprises the nucleotide sequence from position 1 to 2763 shown in SEQ ID NO.5 optionally without the signal peptide-coding section,

(ii) a nucleic acid which comprises a nucleotide sequence corresponding to the nucleic acid from (i) within the scope of the degeneracy of the genetic code and

(iii) a nucleic acid which comprises a nucleotide sequence that hybridizes with the nucleic acids from (i) or/and (ii) under stringent conditions.

40. Process as claimed in one of the ~~claims 37-39,~~ claim 37
w h e r e i n
a further S-layer gene is expressed in the host cell which codes for an unmodified S-layer protein.

41. Process as claimed in claim 40,
w h e r e i n
the unmodified S-layer protein is capable of forming an S-layer structure that is compatible with the recombinant S-layer protein.

42. Process as claimed in one of the ~~claims 37-39,~~ claim 37
w h e r e i n
no further S-layer gene is expressed in the host cell which codes for an unmodified S-layer protein which is capable of forming an S-layer structure that is compatible with a recombinant S-layer protein.

43. Process as claimed in one of the ~~claims 37-42,~~ claim 37
w h e r e i n
a prokaryotic host cell is used.

44. Process as claimed in claim 43,
w h e r e i n
a gram-positive host cell is used.

45. Process as claimed in claim 44,
w h e r e i n
B.stearothermophilus is used.

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